EXHIBIT 1

US005304487A

United States Patent [19]

Wilding et al.

[11] Patent Number: 5,304,487

[45] Date of Patent: Apr. 19, 1994

[54] FLUID HANDLING IN MESOSCALE ANALYTICAL DEVICES

[75] Inventors: Peter Wilding, Paoli; Larry J. Kricka, Berwyn; Jay N. Zemel, Jenkintown, all of Pa.

[73] Assignee: Trustees of the University of Pennsylvania, Philadelphia, Pa.

[21] Appl. No.: 877,536

[22] Filed: May 1, 1992

[51] Int. Cl.[5] G01N 31/00

[52] U.S. Cl. 435/291; 422/55; 422/58; 422/61; 435/7.2; 435/7.21; 436/164; 436/524; 436/809

[58] Field of Search 422/55, 58, 61; 436/524, 164, 807, 809, 49, 501, 180; 435/2, 7.21, 359, 291, 6, 7.2

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4,676,274	6/1987	Brown	422/82
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4,908,112	3/1990	Pace	204/299 R
4,911,782	3/1990	Brown	156/633
4,963,498	10/1990	Hillman et al.	436/69
4,999,283	3/1991	Zavos et al.	435/2
5,114,858	5/1992	Williams et al.	435/259
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5,147,606	9/1992	Charlton et al.	422/56

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4028771	2/1991	Fed. Rep. of Germany .
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91/15750	10/1991	PCT Int'l Appl. .
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Primary Examiner—James C. Housel
Assistant Examiner—Ramon Torres
Attorney, Agent, or Firm—Testa, Hurwitz & Thibeault

[57] ABSTRACT

Devices are provided for analyzing a fluid cell containing sample. The devices comprise a solid substrate, microfabricated to define at least one sample inlet port and a mesoscale flow system. The mesoscale flow system includes a sample flow channel, extending from the inlet port, and a cell handling region for treating cells disposed in fluid communication with the flow channel. The devices may further include a structure inducing flow of cells in the sample through the flow system. In one embodiment, the cell-handling region may comprise a cell lysis structure to enable the lysis of cells in the sample, prior to, e.g., the detection of an intracellular component in the cell sample. In another embodiment, the cell handling region may comprise a cell capture region, comprising binding sites which reversibly bind to a specific population of cells in the cell sample, to permit the isolation of the specific cell population from the sample. The devices can be utilized in a wide range of automated sensitive and rapid tests for the analysis of a fluid cell containing sample.

26 Claims, 7 Drawing Sheets

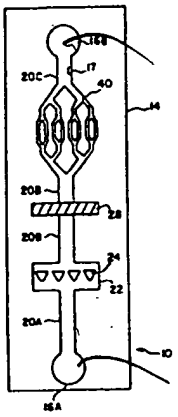

EXHIBIT 2

US005770029A

United States Patent [19]

Nelson et al.

[11] **Patent Number: 5,770,029**

[45] **Date of Patent: Jun. 23, 1998**

[54] **INTEGRATED ELECTROPHORETIC MICRODEVICES**

[75] Inventors: **Robert J. Nelson**, Alameda; **Herbert H. Hooper**, Belmont, both of Calif.; **James Landers**, Rochester, Minn.

[73] Assignee: **Soane Biosciences**, Hayward, Calif.

[21] Appl. No.: **690,307**

[22] Filed: **Jul. 30, 1996**

[51] **Int. Cl.**[6] **G01N 27/26**; G01N 27/447

[52] **U.S. Cl.** **204/604**; 204/453

[58] **Field of Search** 204/453, 604

[56] **References Cited**

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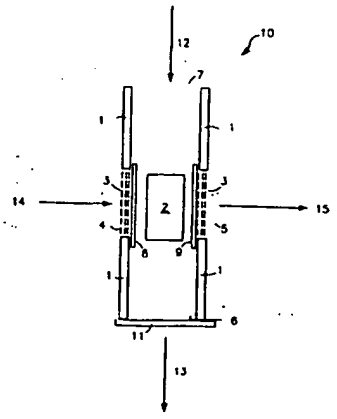
Primary Examiner—Kathryn L. Gorgos
Assistant Examiner—John S. Starsiak, Jr.
Attorney, Agent, or Firm—Flehr Hohbach Test Albritton & Herbert LLP

[57] **ABSTRACT**

Integrated electrophoretic microdevices comprising at least an enrichment channel and a main electrophoretic flowpath are provided. In the subject integrated devices, the enrichment channel and the main electrophoretic flowpath are positioned so that waste fluid flows away from said main electrophoretic flowpath through a discharge outlet. The subject devices find use in a variety of electrophoretic applications, including clinical assays.

14 Claims, 5 Drawing Sheets



EXHIBIT

US005126022A

United States Patent [19]

Soane et al.

[11] Patent Number: 5,126,022

[45] Date of Patent: Jun. 30, 1992

[54] METHOD AND DEVICE FOR MOVING MOLECULES BY THE APPLICATION OF A PLURALIT OF ELECTRICAL FIELDS

[75] Inventors: David S. Soane; Zoya M. Soane, both of Piedmont, Calif.

[73] Assignee: Soane Tecnologies, Inc., Hayward, Calif.

[21] Appl. No.: 487,021

[22] Filed: Feb. 28, 1990

[51] Int. Cl.[5] G01N 27/26

[52] U.S. Cl. ..... 204/180.1; 204/182.8; 204/183.1; 204/299 R; 204/300 R

[58] Field of Search ..... 204/182.8, 299 R, 183.1, 204/300 R, 180.1

[56] References Cited

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Primary Examiner—John Niebling
Assistant Examiner—David G. Ryser
Attorney, Agent, or Firm—Kilpatrick & Cody

[57] ABSTRACT

Devices and methods are disclosed for moving charged molecules through a medium by the application of a plurality of electrical fields of sufficient strength and applied for sufficient amounts of time so as to move the charged molecules through the medium. The devices although preferably small in size, preferably generate large numbers (100 or more) of electrical fields to a movement area which preferably contains a liquid buffered or gel medium. Mixtures of charged molecules are pulled through the gel by the force of the electrical fields. The fields are preferably activated simultaneously or sequentially one after another at various speeds to create complex force field distributions or moving field waves along the separation medium. Charged molecules capable of moving quickly through the gel will be moved along by the faster moving field waves and be separated from slower moving molecules. The fields can be activated by computer software and can be used to move molecules away from and toward each other to obtain rapid and complex chemical synthesis, sequencing or reaction protocols.

21 Claims, 1 Drawing Sheet

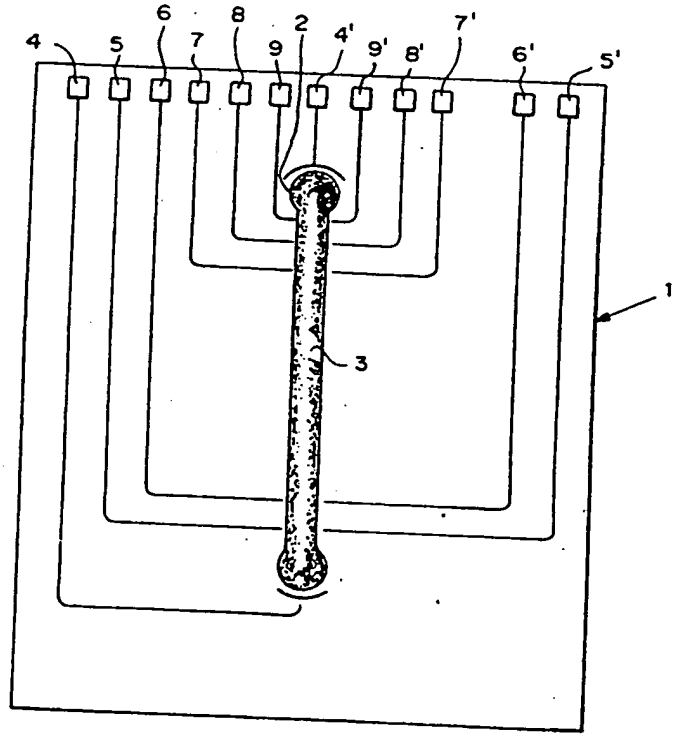

EXHIBIT 4

US005631337A

United States Patent [19]

Sassi et al.

[11] Patent Number: 5,631,337

[45] Date of Patent: May 20, 1997

[54] **THERMOREVERSIBLE HYDROGELS COMPRISING LINEAR COPOLYMERS AND THEIR USE IN ELECTROPHORESIS**

[75] Inventors: **Alexander P. Sassi**, Berkeley; **Shi Lin**, Fremont; **M. Goretty Alonso-Amigo**, Santa Clara; **Herbert H. Hooper**, Belmont, all of Calif.

[73] Assignee: **Soane BioScience**, Hayward, Calif.

[21] Appl. No.: **589,026**

[22] Filed: **Jan. 19, 1996**

[51] **Int. Cl.**[6] **C08F 220/56**; C08F 222/38

[52] **U.S. Cl.** **526/307.2**; 526/306; 526/307.3; 526/310

[58] **Field of Search** 526/306, 307.2, 526/307.3, 310

[56] **References Cited**

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Righetti and Snyder, "Thermally Reversible Gels in Electrophoresis. I: Matrix Characterization," Applied and Theoretical Electrophoresis (1988), 1:53–58.

Primary Examiner—Tae Yoon

Attorney, Agent, or Firm—Flehr Hohbach Test Albritton & Herbert LLP

[57] **ABSTRACT**

Thermoreversible hydrogels comprsing non-ionic, linear copolymers, and methods of their use in electrophoresis, are provided. The subject copolymers comprise polyacrylamide backbones, where a portion of the acrylamide monomeric units compose hydrogen bonding groups as N-substituents. Combination of the subject copolymers with an aqueous phase provides thermoreversible hydrodgels which find use as separation media in electrophoretic applications.

18 Claims, No Drawings

EXHIBIT 5

US005569364A

United States Patent [19]

Hooper et al.

[11] Patent Number: 5,569,364

[45] Date of Patent: Oct. 29, 1996

[54] SEPARATION MEDIA FOR ELECTROPHORESIS

[75] Inventors: Herbert H. Hooper, Belmont; Stephen Pacetti, Sunnyvale; David S. Soane, Piedmont, all of Calif.; Young C. Bae, Scoul, Rep. of Korea

[73] Assignee: Soane Biosciences, Inc., Hayward, Calif.

[21] Appl. No.: 241,048

[22] Filed: May 10, 1994

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 971,956, Nov. 5, 1992, abandoned.

[51] Int. Cl.[6] C25B 9/00

[52] U.S. Cl. 204/455; 204/462; 204/466; 204/468; 204/605; 204/613; 204/616; 252/315.1; 526/303.1; 526/306; 524/555

[58] Field of Search ..... 204/182.8, 299 R; 252/315.1; 526/303.1, 306; 524/555

[56] References Cited

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Primary Examiner—John Niebling
Assistant Examiner—C. Delacroix-Muirheid
Attorney, Agent, or Firm—Bertram I. Rowland, Ph.D.

[57] ABSTRACT

Separation media for electrophoresis, and methods of filling and flushing of electrophoretic devices such as capillaries are described. By preparing submicron to above-micron sized cross-linked gel particles and using gel swelling equilibrium concepts, such devices can be easily filled and flushed. Gel particles can be prepared by inverse suspension, precipitation and suspension polymerization. These particles can be swollen and collapsed by small changes in temperature, pH, and ionic strength of solvent. Other approaches involve the formation of reversible cross-links by use of polyelectrolyte complexes, chelating agents or copolymers of hydrophobic and hydrophilic repeat units. Finally, reversibly solubilized systems may be used to change the viscosity of the media.

24 Claims, 13 Drawing Sheets

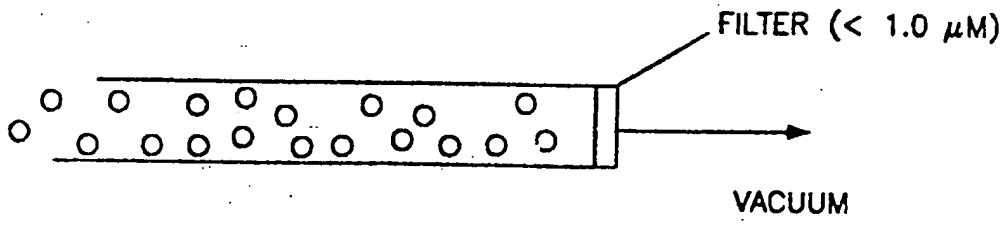

EXHIBIT 6

US005750015A

United States Patent [19]

Soane et al.

[11] Patent Number: 5,750,015

[45] Date of Patent: May 12, 1998

[54] **METHOD AND DEVICE FOR MOVING MOLECULES BY THE APPLICATION OF A PLURALITY OF ELECTRICAL FIELDS**

[75] Inventors: **David S. Soane; Zoya M. Soane**, both of Piedmont, Calif.

[73] Assignee: **Soane Biosciences**, Hayward, Calif.

[21] Appl. No.: **615,642**

[22] Filed: **Mar. 13, 1996**

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 430,134, Apr. 26, 1995, abandoned, which is a continuation of Ser. No. 196,763, Feb. 14, 1994, abandoned, which is a continuation of Ser. No. 880,187, May 7, 1992, abandoned, which is a continuation of Ser. No. 487,021, Feb. 28, 1990, Pat. No. 5,126,022.

[51] **Int. Cl.**[6] **G01N 27/26**; G01N 27/447

[52] **U.S. Cl.** **204/454**; 204/451; 204/547; 204/601; 204/643

[58] **Field of Search** 204/547, 643, 204/454, 451, 601

[56] **References Cited**

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4,474,889	10/1984	Terry et al.	436/161

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Primary Examiner—Kathryn L. Gorgos
Assistant Examiner—John S. Starsiak, Jr.
Attorney, Agent, or Firm—Flehr Hohbach Test Albritton & Herbert LLP; Richard F. Trecartin

[57] **ABSTRACT**

Devices and methods are disclosed for moving charged molecules through a medium by the application of a plurality of electrical fields of sufficient strength and applied for sufficient amounts of time so as to move the charged molecules through the medium. The devices although preferably small in size, preferably generate large numbers (100 or more) of electrical fields to a movement area which preferably contains a liquid buffered or gel medium. Mixtures of charged molecules are pulled through the gel by the force of the electrical fields. The fields are preferably activated simultaneously or sequentially one after another at various speeds to create complex force field distributions or moving field waves along the separation medium. Charged molecules capable of moving quickly through the gel will be moved along by the faster moving field waves and be separated from slower moving molecules. The fields can be activated by computer software and can be used to move molecules away from and toward each other to obtain rapid and complex chemical synthesis, sequencing or reaction protocols.

47 Claims, 2 Drawing Sheets

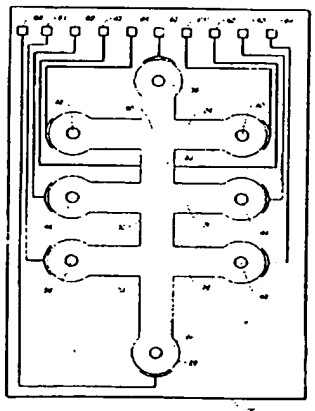

EXHIBIT 7

US005135627A

United States Patent [19]

S ane

[11] Patent Number: 5,135,627

[45] Date of Patent: Aug. 4, 1992

[54] MOSAIC MICROCOLUMNS, SLABS, AND SEPARATION MEDIA FOR ELECTROPHORESIS AND CHROMATOGRAPHY

[75] Inventor: David S. Soane, Piedmont, Calif.

[73] Assignee: Soane Technologies, Inc., Hayward, Calif.

[21] Appl. No.: 597,528

[22] Filed: Oct. 15, 1990

[51] Int. Cl.[5] C25B 1/00; B01D 57/02; B01D 61/42

[52] U.S. Cl. ..... 204/182.8; 204/180.1; 204/299 R; 428/327

[58] Field of Search ..... 204/182.8, 180.1, 299 R; 428/327

[56] References Cited

U.S. PATENT DOCUMENTS

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4,911,807	3/1990	Burd .	
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Brown and Pietrzyk, *J. Chromatography* 466, 291–300 (1989).

Issaq, et al., *J. Liquid Chromatography* 11(2), 333–348 (1988).

Primary Examiner—John Niebling
Assistant Examiner—Caroline Koestner
Attorney, Agent, or Firm—Kilpatrick & Cody

[57] ABSTRACT

A method and compositions for separating molecules based on molecular size, shape, affinity, chirality, weight, charge, and hydrogen bonding, using a mosaic matrix formed by polymerizing a dispersion of dispersoids within a polymeric matrix. The dispersoids and matrix can be of the same or different hydrophobicity or hydrophilicity. The dispersoids can be porous or non-porous. The mosaic matrix can be used with existing chromatographic and electrophoresis apparatus to effect an enhanced separation of molecules, particularly of nucleic acids and peptides, by application of a solution and/or an electrical field to the matrix. The solution can form a pH, ionic, or composition gradient, and be applied using gravity or under pressure. The electrical field can be continuous, pulsed, or two-dimensional.

33 Claims, 1 Drawing Sheet

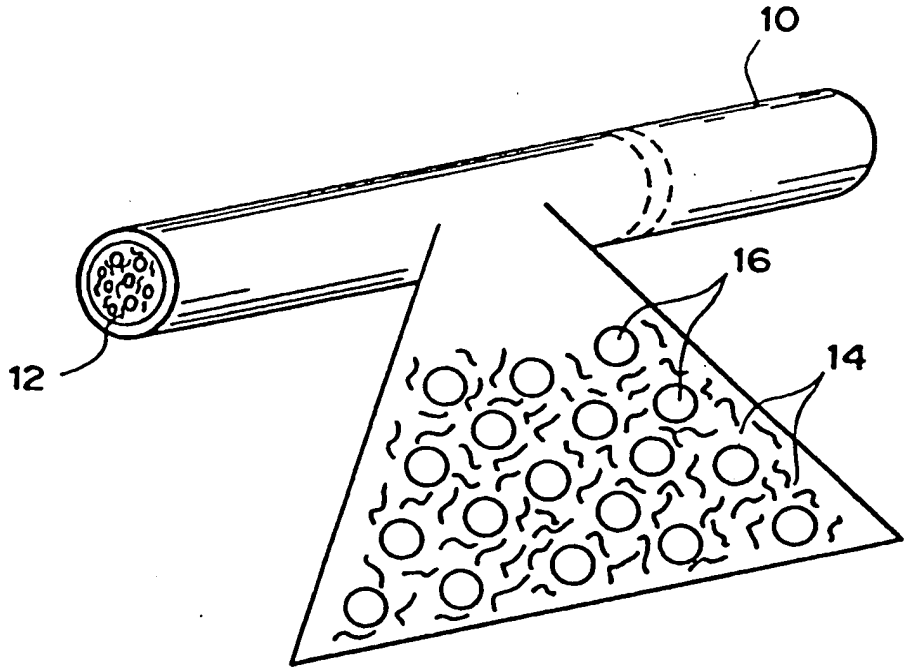

EXHIBIT 8

US005498392A

United States Patent [19]

Wilding et al.

[11] Patent Number: 5,498,392

[45] Date of Patent: Mar. 12, 1996

[54] MESOSCALE POLYNUCLEOTIDE AMPLIFICATION DEVICE AND METHOD

[75] Inventors: Peter Wilding, Paoli; Larry J. Kricka, Berwyn, both of Pa.

[73] Assignee: Trustees of the University of Pennsylvania, Philadelphia, Pa.

[21] Appl. No.: 308,199

[22] Filed: Sep. 19, 1994

Related U.S. Application Data

[63] Continuation of Ser. No. 877,662, May 1, 1992, abandoned.

[51] Int. Cl.[6] G01N 25/00; G01N 33/50; C12P 19/34

[52] U.S. Cl. 422/68.1; 422/50; 422/55; 422/61; 422/62; 422/63; 422/82.05; 422/102; 435/6; 435/91.1; 435/91.2; 435/285.1; 435/285.2; 435/810; 436/807; 935/78; 935/88

[58] Field of Search 435/6, 91.1, 91.2, 435/288, 291, 316, 803, 810; 436/807; 536/22.1, 23.1, 24.1; 935/78, 88; 422/50, 55, 61, 62, 63, 68.1, 82.05, 102

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4,790,640	12/1988	Nason	350/534
4,886,761	12/1989	Gustafson et al.	436/518
4,908,112	3/1990	Pace	204/299 R
4,911,782	3/1990	Brown	156/633
4,963,498	10/1990	Hillman et al.	436/69
5,135,720	8/1992	Uchida	422/107
5,147,606	9/1992	Charlton et al.	422/56
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Primary Examiner—W. Gary Jones
Assistant Examiner—Ardin H. Marschel
Attorney, Agent, or Firm—Dann, Dorfman, Herrell and Skillman

[57] ABSTRACT

Disclosed are devices for amplifying a preselected polynucleotide in a sample by conducting a polynucleotide polymerization reaction. The devices comprise a substrate microfabricated to define a sample inlet port and a mesoscale flow system, which extends from the inlet port. The mesoscale flow system includes a polynucleotide polymerization reaction chamber in fluid communication with the inlet port which is provided with reagents required for polymerization and amplification of a preselected polynucleotide. In one embodiment the devices may be utilized to implement a polymerase chain reaction (PCR) in the reaction chamber (PCR chamber). The PCR chamber is provided with the sample polynucleotide, polymerase, nucleoside triphosphates, primers and other reagents required for the polymerase chain reaction, and the device is provided with means for thermally controlling the temperature of the contents of the reaction chamber at a temperature controlled to dehybridize double stranded polynucleotide, to anneal the primers, and to polymerize and amplify the polynucleotide.

27 Claims, 11 Drawing Sheets

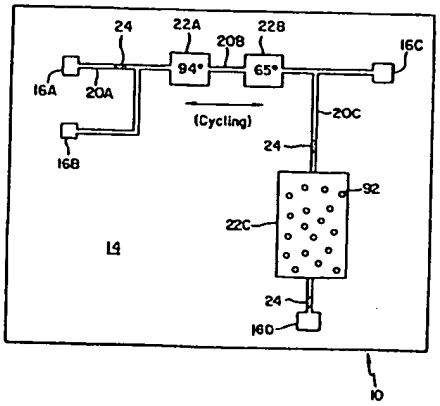

EXHIBIT 9

US005587128A

United States Patent [19]

Wilding et al.

[11] Patent Number: 5,587,128

[45] Date of Patent: *Dec. 24, 1996

[54] MESOSCALE POLYNUCLEOTIDE AMPLIFICATION DEVICES

[75] Inventors: Peter Wilding, Paoli; Larry J. Kricka, Berwyn, both of Pa.

[73] Assignee: The Trustees of the University of Pennsylvania, Philadelphia, Pa.

[*] Notice: The term of this patent shall not extend beyond the expiration date of Pat. No. 5,304,487.

[21] Appl. No.: 338,728

[22] Filed: Nov. 14, 1994

Related U.S. Application Data

[63] Continuation-in-part of Ser. No. 308,199, Sep. 19, 1994, Pat. No. 5,498,392, which is a continuation of Ser. No. 877,662, May 1, 1992, abandoned.

[51] Int. Cl.[6] C12Q 1/68; G01N 33/50; G01N 21/01

[52] U.S. Cl. 422/50; 422/54; 422/55; 422/56; 422/57; 422/58; 422/68.1; 422/69; 422/82.01; 422/82.02; 422/82.05; 422/82.06; 422/82.07; 422/82.08; 422/82.09; 422/129; 422/131; 422/138; 435/6; 435/90; 435/91.1; 435/91.2; 435/91.3; 435/91.5; 435/91.51; 435/173.1; 435/174; 435/176; 435/177; 435/283.1; 435/285.1; 435/285.2; 435/287.1; 435/287.2; 435/287.3; 435/287.7; 435/287.8; 435/287.9; 435/288.7; 435/289.1; 435/290.1; 435/292.1; 435/299.1; 435/808; 435/810; 435/814; 436/518; 436/524; 436/525; 436/528; 436/531; 436/532; 436/535; 436/63; 436/164; 436/165; 436/166; 436/169; 436/172; 436/175; 437/1; 437/5; 437/51; 437/61; 437/180; 437/181; 437/189; 437/225; 437/946; 536/22.1; 536/23.1; 935/77; 935/78; 935/88

[58] Field of Search 422/50, 54, 55, 422/56, 57, 58, 68.1, 69, 82.01, 82.02, 82.05–.09, 129, 131, 138; 435/91.2, 91.1, 6, 5, 291, 810, 90, 91, 91.3, 91.5, 91.51, 173.1, 174, 176, 177, 283.1, 285.1, 285.2, 287.1–.3, 287.7–.9, 288.7, 289.1, 290.1, 292.1, 299.1, 808, 814; 437/1, 5, 51, 61, 180, 181, 189, 225, 946; 536/22.1, 23.1, 25.3; 935/77, 78, 88; 436/518, 524, 525, 528, 531, 532, 535, 63, 164–6, 169, 172, 175, 805

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Attorney, Agent, or Firm—Dann, Dorfman, Herrell and Skillman

[57] ABSTRACT

Disclosed are devices for amplifying a preselected polynucleotide in a sample by conducting a polynucleotide amplification reaction. The devices are provided with a substrate microfabricated to include a polynucleotide amplification reaction chamber, having at least one cross-sectional dimension of about 0.1 to 1000 μm. The device also includes at least one port in fluid communication with the reaction chamber, for introducing a sample to the chamber, for

(Abstract continued on next page.)